SCIENTIFIC LETTER

Detection of functional differences between different platelet membrane glycoprotein Ibα variable number tandem repeat and Kozak genotypes as shown by the PFA-100 system

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The interaction between the platelet membrane glycoprotein Ib/IX/V and von Willebrand factor (VWF) is the primary event in the formation of an occlusive thrombus under conditions of high shear stress.[1] Several polymorphisms of glycoprotein Ibα (the functional binding site for surface bound VWF) have been reported.[2] The relation of variable number tandem repeat (VNTR) and Kozak sequence polymorphisms with arterial thrombosis and coronary artery disease remains controversial. In our recent study we proposed that the glycoprotein Ibα genotypes CC of the VNTR and Kozak −5TT increased the risk of platelet plug formation under the condition of high shear stress generated by stenosed vessels and thus caused the increased risk of myocardial infarction (MI), which we observed in a group of patients with known coronary artery disease status who had those genotypes. We also observed the potential protective effect of the glycoprotein Ibα Kozak −5TC genotype.[3] The objective of this study was to evaluate the effect of these polymorphisms on platelet function of the same group of patients by using the platelet function analyser system PFA-100 (Dade Behring), which mimics physiological conditions in vivo involving platelet binding to VWF and platelet adhesion/aggregation in response to collagen under conditions of high shear after activation by adrenaline (epinephrine) or ADP.

METHODS

From 256 patients who had previously been genotyped for the VNTR and Kozak sequence polymorphisms, 84 white men were consecutively selected (mean age 62.07 years). All the patients were taking aspirin 75 mg as their only antiplatelet medication. Patients who had an MI within the previous six months and those with bleeding diathesis, abnormal platelet counts, or abnormal haemoglobin were excluded from the study. A fasting venous blood sample was obtained between 8 am and 10 am with a 21 gauge butterfly cannula without a tourniquet. The blood was used for the VWF assay, full blood count, and platelet function analysis.

Genomic DNA was extracted from whole blood by using the BACC2 kit (Nucleon Biosciences, Coatbridge, Lanarkshire, UK). Glycoprotein Ibα VNTR and Kozak sequence polymorphisms were genotyped as previously described.[3]

The citrated blood was allowed to stand for 30–60 minutes before platelet reactivity was measured with the PFA-100. The maximum recordable closure time is 300 seconds. Plasma VWF antigen concentration was measured by enzyme linked immunosorbent assay (ELISA) with a rabbit monoclonal antibody to VWF (DAKO Ltd, High Wycombe, UK). The optical density was measured at 492 nm with a Dynatech MR 700 ELISA plate reader (Dynatech Laboratories Inc, Chantilly, Virginia, USA). The normal range was 0.5–2.0 U/ml.

The published coefficient of variation of the PFA-100 in normal subjects ranges from 3–18% with average variability of 8.5%.[4] In our study, because all the patients were taking aspirin, we selected 12 healthy male volunteers with no bleeding disorder and normal blood count and VWF antigen concentrations. We instructed them to take aspirin 75 mg daily. Their blood was sampled out under the same conditions as above. The closure time for collagen/adrenaline in 95% of these selected participants was 100–200 seconds and for collagen/ADP, 80–120 seconds. Therefore, we used these values as the normal range with aspirin medication for the purpose of this study.

Associations were analysed with the statistical package SPSS version 12 (SPSS Inc, Chicago, Illinois, USA). To compare the groups for conventional cardiac risk factors in different genotype groups we used the χ^2 or Fisher's exact test. The effect of age was analysed as a single continuous variable by using linear regression analysis of variance. The influence of genotype was assessed with the Pearson χ^2 test. Values of $p < 0.05$ were considered to be significant.

RESULTS

The study population consisted of 84 male patients with the following genotypes: 30 VNTR-CC, 28 VNTR-BC, 26 VNTR-CD, 52 Kozak-TT, and Kozak-TC. The MI and non-MI groups did not differ in the number of patients with hypercholesterolaemia, diabetes, smoking history, and hypertension. Analysis of platelet function in the MI group showed a marginal increase in platelet reactivity compared with the non-MI patients. More non-MI patients appeared to have a closure time greater than 200 seconds (38.9%) compared with only 14.3% with a closure time less than 100 seconds ($p = 0.07$). A significant difference was seen between the VNTR genotypes in response to collagen/adrenaline: 43.3% with the CC genotype had a closure time less than 100 seconds compared with 69.2% with the CD genotypes with a closure time more than 200 seconds ($p = 0.0001$). Twice as many patients with the Kozak −5TC genotype had a collagen/adrenaline closure time more than 200 seconds compared with the TT genotype (46% *v* 23.1%, $p = 0.05$).

We also found similar results comparing the means of the closure time in the subgroups (fig 1A). The closure time in patients with minor or single vessel disease was not different from the closure time in patients with more than one vessel disease (186.31 (84.48) *v* 164.38 (79.63), $p = 0.225$ with collagen/adrenaline). Although hypertension, diabetes mellitus, and smoking had no effect on the degree of vessel disease, the serum cholesterol concentration was strongly correlated with the degree of vessel disease ($p = 0.0001$).

Abbreviations: ELISA, enzyme linked immunosorbent assay; MI, myocardial infarction; VNTR, variable number tandem repeat; VWF, von Willebrand factor

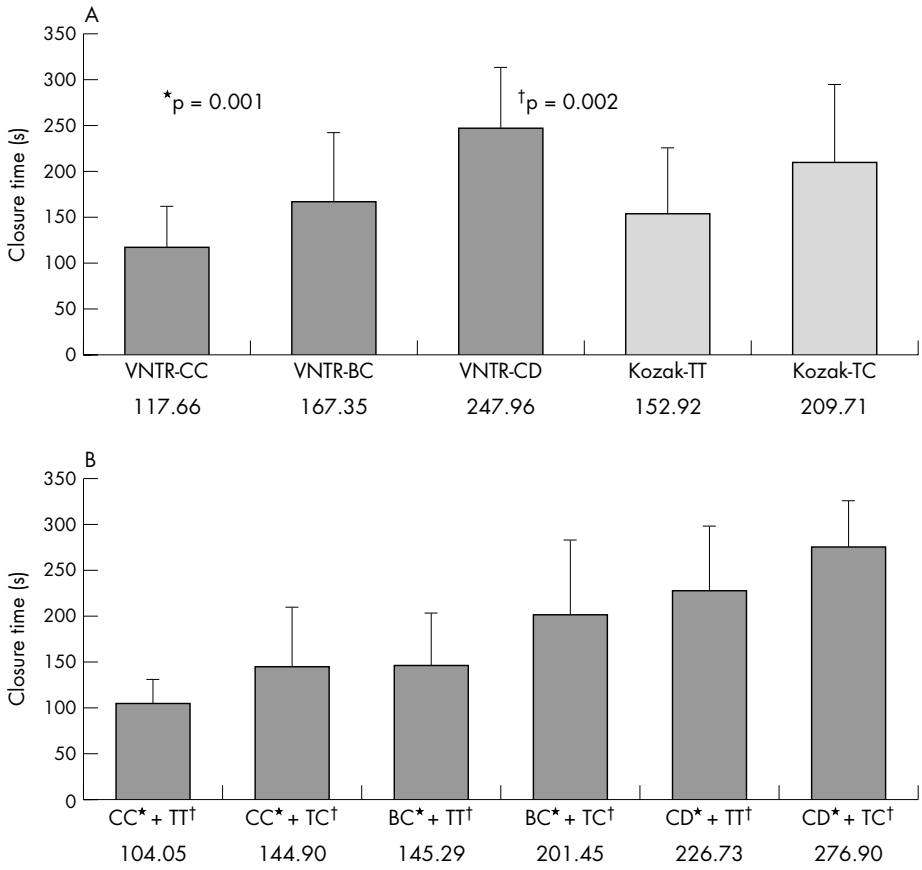

Figure 1 Mean closure time (in seconds) in response to collagen/adrenaline in variable number tandem repeat (VNTR) and Kozak sequence polymorphisms measured by PFA-100. (A) *Comparison of VNTR subgroups; †Comparison of Kozak sequence polymorphism subgroups. (B) Mean closure time in combination of polymorphisms. *VNTR polymorphism; †Kozak polymorphism, p = 0.002, χ^2 = 10.5, odds ratio 1.14 (95% confidence interval 3.38 to 0.98).

The collagen/ADP channel did not detect any difference between any of the subgroups. There was no significant difference in the plasma concentration of VWF between all five genotypes and no correlation between the VWF antigen concentration and the closure time (p = 0.125). The combination of the VNTR-CC genotype with the Kozak −5TT sequence polymorphism had the shortest closure time and the combined VNTR-CD and Kozak −5TC polymorphism had the longest closure time when the collagen/adrenaline cartridge was used (104.05 (26.20) *v* 276.9 (50.10) seconds, respectively, p = 0.002) (fig 1B).

DISCUSSION

This follow up study was stimulated by our earlier observation that the difference in risk associated with VNTR and Kozak sequence polymorphisms appeared to be more closely linked to thrombosis than to atheroma.[3] In the present study we found no correlation between the severity of coronary artery disease and platelet function. This is consistent with the finding that, in patients with stable angina, the degree of luminal narrowing does not predict the likelihood of future MI.[5]

In this study we found a marginal increase in platelet reactivity in patients with a history of acute MI. We showed that more patients with MI than without MI had a closure time less than 100 seconds, although this did not reach significance. This can be explained by the relatively small sample size.

In this study we have selected only male white patients to enable differences in platelet reactivity based on genotype to be identified. Knowing the patients' coronary artery status and minimising the factors that can affect platelet function (diurnal variability, smoking, VWF concentration, etc) has helped us to detect the effect of these polymorphisms on platelet function. Genotypic variation in the glycoprotein Ibα receptor may have functional significance, perhaps resulting in increased binding to VWF. In contrast to earlier studies[6 7] in our study the VNTR-CC genotype (two tandem repeat) is associated with increased platelet reactivity. We can hypothesise that a physically shorter receptor that brings the platelet closer to the subendothelial surface can result in more efficient binding and subsequently marginally increase the risk of coronary event. In our earlier study we showed that the combined VNTR-CD and Kozak −5TC genotype was protective against MI.[3] That observation is now further supported in this functional study, as patients with the combined VNTR-CD and Kozak −5TC genotype have the longest closure time, thus conferring lower risk. A glycoprotein Ib/IX/V receptor that has a heterozygous glycoprotein Ibα genotype as in the VNTR-CD may be less favourable for efficient binding to surface bound VWF on damaged subendothelium under shear due to the unequal lengths of the glycoprotein Ibα glycoprotein. This is supported by the increased closure times seen with the VNTR-CD genotype in this study.

We recognise the limitations of this pilot study. Our study population is small and did not include the less frequent VNTR A allele (common in the Japanese population) and less frequent homozygous Kozak −5CC genotype. A larger study will be required to fully assess the true implications of these genotypes in clinical practice and for recognition of high risk patients with coronary artery disease.

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IMAGES IN CARDIOLOGY

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Multiple complications of aortic valve endocarditis diagnosed from the ECG

The ECG is often overlooked as a diagnostic tool in conditions other than ischaemic heart disease and arrhythmias. A 48 year old man presented with symptoms of fever, headache, malaise, and abdominal pain. He was apyrexial but was admitted for further investigation. His presenting ECG was unremarkable, showing sinus tachycardia but no other abnormal features. Twenty four hours later, blood cultures grew *Staphylococcus aureus* and the patient was referred for cardiology review at the suggestion of the microbiologists. The patient complained of no new symptoms. The ECG taken at this time showed tachycardia; it also showed notable PR interval prolongation and widespread ST elevation consistent with pericarditis. Consequently, a provisional diagnosis of aortic valve endocarditis with root involvement and pyopericardium was made based on the ECG findings. Subsequent clinical and echocardiographic examination confirmed severe aortic regurgitation and pericardial effusion, and the patient was taken to theatre. Operative findings were of free aortic regurgitation with pus tracking around the commissure between the non- and right coronary cusps and into the pericardium. The patient went on to have a successful aortic valve replacement and antimicrobial treatment. This case acts as a reminder of the diagnostic ability of the ECG, of the rapidly progressive course of staphylococcal endocarditis, and of the importance of daily ECG examination in patients with suspected aortic valve endocarditis.

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